## REMARKS

Applicants have amended the claims to more particularly define the invention taking into consideration the outstanding Official Action. Applicants note that Applicants election without traverse of claims 45-56 and 61 in the reply filed on 6/30/08 is acknowledged and that claims 57-60 are withdrawn from further consideration as being drawn to a non-elected invention, there being no allowable generic or linking claim present. Applicants have indicated claims 57-60 as withdrawn from consideration in the present application. Applicants retain their right to file a further application at a later time.

Applicants have amended claims 45, 51-52 and 61 and have canceled claims 47 and 55 from the present application without prejudice or dislciamer . Applicants have added new claim 62 to the present application.

In this regard, the claims have been amended to refer to specific embodiments of the invention, i.e. methods and cell populations obtainable by said methods, which are characterized by using the specific combination of one or more ligands of the gp130 receptor and one or more ligands of the EGF receptor, wherein one of said one or more ligands of the EGF receptor is EGF.

Claims 45 and 52 have been amended to refer specifically to an in vitro method and a cell population obtainable by said method, respectively, said method comprising the addition of and incubation with a combination of one or more ligands of the gp130 receptor and one or more ligands of the EGF receptor, wherein one of said one or more ligands of the EGF receptor is EGF.

Support for the amendments to claims 45 and 52 can be found in previous claims 45, 47 and 52 and on p. 2 [0011] l. 5-9 of the published US application, describing that: *"In a particular embodiment, the method is performed by adding in step b) both one or more ligands of the gp130 receptor of a second mammal as well as adding one or more ligands of the EGF receptor of a third mammal to said culture medium"*. It is also stated on p. 3 [0022] of the published US application that: *"...this is done by incubating said dedifferentiated pancreatic cells in a medium comprising a ligand of the EGF receptor, for example EGF..."*

For the sake of clarity, claim 51 has been amended to recite *"a preliminary step of depleting beta cells from said population"* in stead of *"a preliminary step of depleting said population from beta cells."*

For reasons of clarity, claim 61 has been amended to recite: "an in vitro method for determining the degree of redifferentiation of a population of mammalian pancreatic cells according to claim 52".

Support for the amendment to claim 61 can be found in previous claim 61 and on p. 3 [0021] l. 1-10 of the published US application:

*"In yet another embodiment the invention relates to an in vitro method for determining the degree of redifferentiation of dedifferentiated mammalian pancreatic cells comprising the steps of determining one or more parameters selected from the group consisting of a) The presence of CK20, CK7 or CK19, b) the occurrence of binucleated cells, c) the presence of insulin positive cells, d) the presence of C-peptide, Pdx-1 and Glut-2, e) the presence of gastrin CCKB receptor, PGP9.5 and notch-1 receptor on said mammalian pancreatic cells."*

With respect to new claim 62, support for this new claim can be found on p. 5 [0043] of the published US application:

*"In one embodiment of the present invention, dedifferentiated cells derived were cultured as monolayers attached to plastic, in the presence of low serum-concentration (1% FBS). Many alternative conditions can be envisaged for the cultivation of dedifferentiated cells of mammals, such as the use of suspension cultures, the use of serum of other animals apart from bovine serum, the use of alternative basis media other than RPMI-1640 and varying glucose concentrations."*

The observations of the Applicant towards the Examiner's objections stated in the Official Action are given below in respect of the claims as currently amended. Applicants submit that all of the claims now present in the application are fully supported by the specification as originally filed and no new matter is introduced.

The objection to claim 61 because of the informalities set forth on page 2 of the outstanding Official Action has been obviated in view of the amendment to the claim. This is similarly true with respect to the objection to claim 51 for the reasons set forth

on page 2 of the outstanding Official Action. The linguistic error of claim 61 has appropriately been corrected by deleting the article "a" in claim 61. Further, Applicant has amended claim 51 in accordance with the Examiner's suggestion. Accordingly, it is most respectfully requested that these objections be withdrawn.

The rejection of claim 61 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention has been carefully considered but is most respectfully traversed in view of the amendments to the claim.

This is similarly true with respect to the 101 rejection of claim 61 for the reasons set forth on page 3 of the outstanding Official Action. Applicant has amended claim 61 to refer to an in vitro method for determining the degree of redifferentiation of a population of mammalian pancreatic cells. Accordingly, it is most respectfully requested that these rejections be withdrawn.

The rejection of claim 55 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention has been carefully considered but is most respectfully traversed in view of the cancellation of this claim for the present application. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 45-46 and 61 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention has been carefully considered but is most respectfully traversed in view of the amendments to the claims. Applicant has amended claims 45 and 52 to refer to "adding one or more ligands of the gp130 receptor of a second mammal and adding one or more ligands of the EGF receptor of a third mammal to said culture medium", rendering the objection moot. Accordingly, it is most respectfully requested that this rejection be withdrawn.

Applicants most respectfully submit that all of the claims now present in the application are in full compliance with 35 USC 112 and clearly patentable over the references of record.

The rejection of claims 45-48, 50, 52 and 61 under 35 USC 103(a) as being

unpatentable over Presnell has been carefully considered but is most respectfully traversed in view of the amendments to the claims.

Applicant respectfully requests reconsideration of this rejection in light of the present claim amendments. Indeed, the claims as presently amended refer to an in vitro method and a cell population obtainable by this method, wherein the method comprises the addition of and incubation with a specific combination of one or more ligands of the gp130 receptor and one or more ligands of the EGF receptor, wherein one of said one or more ligands of the EGF receptor is EGF.

Presnell et al. generally teach methods for expansion and transdifferentiation of pancreatic acinar cells to insulin-producing cells in vitro. These methods comprise a first phase of dedifferentiation of the acinar cells to an intermediate precursor (IP) cell phenotype, expressing a number of ductular cytokeratins (CK7, CK19) and a second phase of differentiation of IP cells into insulin-producing cells.
Indeed, it is stated by Presnell et al. on p. 1-2:

> *[0009] The present invention provides compositions and methods whereby, e.g., acinar cells can be cultivated successfully in vitro, undergoing a 3-4 fold increase in cell number over time, and giving rise to a cell population that co-expresses acinar and ductal markers early during the culture (2-3 days ex vivo), then ultimately (e.g., about 7-8 days ex vivo) acquires a modified phenotype characterized by expression of some acinar-associated genes, as well as some liver-associated genes. The genes expressed by these modified cells at about 7-8 days ex vivo include, e.g., ductular cytokeratins (CK7, CK8, CK18 and CK19), hepatic nuclear factor 1 (HNF1), alpha-1 antitrypsin, pi-glutathione s transferase (pi-GST), liver-specific (basic helix-loop-helix (bHLH) transcription factor, Thy-1, CCAAT/enhancer-binding protein (C/EBP)-alpha and C/EBP-beta. These cells exhibit little if any expression of the pancreas-associated genes carbonic anhydrase, cystic fibrosis transmembrane conductance regulator (CFTR), elastase and amylase. By "little if any" expression of a gene is meant herein that gene expression is generally undetectable under conventional methods, such as the hybridization and immunocytochemical methods described herein, but expression may be detected by extraordinarily sensitive methods, such as PCR-based analysis. This type of modified cell is referred to herein as an intermediate progenitor ("IP") cell. The expanded/transdifferentiated acinar cells (IP cells) can be produced using a general serum-containing media, or, in a*

> *preferred method, can be produced without serum on a surface comprising one or more extracellular matrix molecules (ECMs) in the presence of one or more soluble active factors. ECMs can be presented in 2 dimensional or 3 dimensional culture systems in the presence of soluble active factors.*
> *[0010] The IP cells generated from these cultures are expected to be useful directly in certain medical applications. For example, there is evidence that such cells may under certain conditions become functioning insulin-producing cells when implanted in diabetic patients. The cells can also be used for drug discovery and toxicity studies.*
> *[0011] In addition, according to a further aspect of the invention, the IP cells can be cultivated further, in a serum-free medium composed of any standard serum-free base medium (DMEM:HamsF12, for example) with BSA and combinations of factors, including ECMs, small molecules, and growth factors. After 5-10 days of culture, the IP cells undergo additional steps of differentiation, culminating in the formation of cell aggregates that express pro-insulin and C-peptide.* (emphasis added)

Presnell et al. further disclose on p. 2 that for the induction of expansion and transdifferentiation of primary pancreatic cells into IP cells, growth factors, such as EGF-receptor activators can be used, whereby EGF and TGFα are particularly preferred:

> *[0015] Soluble active factors for the expansion and transdifferentiation of primary pancreatic acinar cells into IP cells include growth factors such as HGF receptor activators and EGF receptor activators. Preferred soluble active factors include one or more of EGF and Transforming Growth Factor-[alpha], IGF1, HGF, betacellulin, prolactin and gastrin 1. HGF, EGF and/or Transforming Growth Factor-[alpha] are particularly preferred. Also preferred is the combination of IGF1 and betacellulin.*

Presnell et al. further disclose on p. 3 a second culture system which promotes transformation of glandular epithelial cells (IP cells) into insulin-producing cells and indicates that this further medium can comprise at least one differentiation promoting factor that promotes the transformation of glandular epithelial cells into insulin-producing cells. Examples of such differentiation promoting factor include LIF and TGF-alpha:

> *[0025] In a second aspect, the invention also provides methods and*

> *compositions for transforming glandular epithelial cells that have acquired expression of markers characteristic of an intermediate progenitor (IP) phenotype as described above into insulin-producing cells.[...]*
> *[0026] In this aspect, the invention provides a second cell culture system comprising a cell attachment surface and a culture medium that supports and promotes the transformation of glandular epithelial cells into insulin-producing cells. The cell attachment surface is similar to and may be identical to the attachment surface for expanding primary pancreatic acinar cells. It may be presented in the form of a flat surface coated on a vessel or in the form of a scaffold or other surface adapted for cell culture. It can be comprised of, or coated with, any composition that is capable of maintaining cells or supporting cell growth. In a preferred embodiment, it comprises at least one ECM, such as Collagen I, Collagen VI, Collagen IV, Vitronectin or Fibronectin. In a particularly preferred embodiment, the cell attachment surface is Collagen-I.*
> *[0027] In this aspect, the invention provides a further culture medium comprising at least one differentiation promoting factor ("DPF") that promotes the transformation of glandular epithelial cells into insulin producing cells. The DPFs for the transformation of glandular epithelial cells into insulin producing cells can be one or more of Activin A, acidic FGF, basic FGF, C-Natriuretic Peptide (CNP), Calcitonin Gene Related Peptide, Cholera Toxin B Subunit, Dexamethasone, Gastrin-Releasing Peptide, Glucagon-like Peptide-1 (GLP-1), Glucose, IGF1, IGF2, Insulin, Laminin, LIF, Met-Enkephalin, PDGFAA+PDGFBB, Prolactin, Sonic Hedgehog, Substance P, TGF-alpha, Trolox (alpha-tocopherol derivative), or VEGF. Preferred concentrations in culture medium of each of these 23 DPFs are listed in Table 1. Although in some cases one DPF is sufficient, preferably two or more factors are used. As many as all 23 of the factors may be used* (emphasis added).

With regard to the latter aspect disclosed in Presnell et al., combinations of factors for use in the 'second phase' of culture are disclosed in paragraph [0093], more specifically a medium comprising the combination of Activin A, CGRP-alpha, Cholera Toxin B, Dexamethasone, Glucose, GLP-1, Insulin, LIF, Laminin, Met-Enkephalin, PDGFAA/BB, Sonic Hedgehog, Substance P, TGF-alpha, aFGF, and VEGF.

Accordingly, it is submitted that Presnell et al. do not envisage the use of EGF and a ligand of the gp130 receptor, such as LIF, in the same cultivation medium. Indeed, it is submitted that Presnell et al. disclose the use of EGF only in the context of dedifferentiation of acinar pancreatic cells. There is no suggestion that the combination of EGF and a gp130 receptor ligand, such as LIF, allows a higher yield of

insulin secreting cells starting from dedifferentiated pancreatic cells. Moreover, it is submitted that, while Presnell et al. disclose a medium comprising both LIF and and TGF-alpha for the differentiation of dedifferentiated pancreatic cells, there is no suggestion in Presnell et al. that the specific combination of TGF-alpha and LIF increases the yield of insulin-producing cells, nor any suggestion to replace TGF-alpha in this combination with another EGF receptor ligand. Indeed, Presnell et al. disclose another combination of factors which include LIF (i.e. combination 2), which does not comprise a gp130 receptor ligand. Accordingly, it is submitted that there is no suggestion in Presnell et al. to replace TGF-alpha by another EGF receptor ligand, let alone with EGF.

The inventors of the present application have found that a specific combination of growth factors comprising both EGF and at least one ligand of the gp130 receptor makes it possible to rapidly obtain a large number of insulin-producing cells from a population of dedifferentiated pancreatic cells (see p. 1 [006], last sentence; p. 3 [0025]; Example 3, in particular p. 7 [0061] of the published application). This finding is not suggested anywhere in Presnell et al. Accordingly, it is submitted that claims 45-48, 50 and 52 as currently amended are non-obvious over Presnell et al. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 45, 47, 50-52, 55, 56 and 61 under 35 USC 103(a) as being unpatentable over Bonner-Weir et al. has been carefully considered but is most respectfully traversed in view of the amendments to the claims.

Applicant again respectfully requests reconsideration of this objection in light of the present claim amendments. The amended claims refer to an in vitro method, comprising the addition of and incubation with a specific combination of one or more ligands of the gp130 receptor and one or more ligands of the EGF receptor, wherein one of said one or more ligands of the EGF receptor is EGF.

Bonner-Weir et al. generally teaches methods of promoting dedifferentiation of pancreatic cells and methods for obtaining pancreatic islet cells from the dedifferentiated pancreatic cells (see abstract). The patent further discloses the

differentiation of dedifferentiated cells in the presence of EGF (p. 2, right column, l. 36-42).

Bonner-Weir et al. does not teach or suggest the claimed methods comprising culturing dedifferentiated pancreatic cells on medium that contains, in addition to EGF, one or more ligands of the gp130 receptor. There is no suggestion in the '203 patent to combine EGF with a ligand of the gp130 receptor in order to obtain a high number of insulin-producing cells. Thus, it is submitted that the '203 patent in no way suggests the presently claimed methods or cell populations. Accordingly, it is most respectfully requested that this rejection be withdrawn.

In view of the above comments and further amendments to the claims, favorable reconsideration and allowance of all the claims now present in the application are most respectfully requested.

Respectfully submitted,

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